RESEARCH ARTICLE

Osteocalcin as a Biomarker for Estimation of Infertility for Iraqi Patients

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ABSTRACT

Objective: The evaluation of serum osteocalcin (OSN) for Iraqi infertile patients to see the effect of osteocalcin insufficiency, which may lead to a decreased level of testosterone production in males that may cause infertility.

Methods: Forty two newly diagnosed infertile males age range (24–47) years and thirty two apparently healthy males as controls age range (25–58) years. Serum levels of testosterone (TEST), stimulating follicle hormone (FSH) and luteinizing hormone (LH), prolactin (PROL), osteocalcin OSN, and fasting blood sugar (FBS) were performed in both patients and controls. Estimation of serum OSN by Immulite1000 auto-analyzer, TEST, FSH, LH, PROL, and FBS by Immulite2000 auto-analyzer.

Results: Infertile patients show significantly elevated serum levels for follicle-stimulating hormone (FSH), LH, and PROL as compared with controls according to p-value (0.000), (0.044), and (0.000), respectively. On the other hand, the infertile patients have lowered serum levels for OSN and TEST as compared with controls according to p-value (0.000) and (0.000), respectively. Fasting blood sugar (FBS) is evaluated within the normal levels for both patients and control groups.

Conclusion: Osteocalcin, act as a regulator of male fertility via its role in the biosynthesis of testosterone, so it serves as a biomarker for evaluation of male infertility. Therefore, osteocalcin could be used as a therapeutic for the treatment of disorders related to male reproduction, including male infertility and low testosterone levels.

Keywords: FSH, LH, Male infertility, Osteocalcin, Prolactin, Testosterone.

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INTRODUCTION

Osteocalcin (OSN), known as "bone gamma-carboxyglutamic acid (Gla) protein", is the most abundant non-collagenous protein of bone matrix.[1] It undergoes posttranslational modifications within the osteoblast before its secretion to blood circulation by the carboxylation of three glutamic residues in positions 17, 21, and 24 in glutamic acid,[2] resulting in a peptide with high affinity toward bone and the extracellular matrix.[3] The low pH inside the osteoclast (resorption compartments), causes the OSN to be decarboxylated again, which diminishes its affinity for bone and begin the release of uncarboxylated OSN into the circulation during bone resorption.[4] Murine and *in vitro* studies, indicate that the uncarboxylated form of OSN controls physiological pathways in an endocrine manner such as glucose homeostasis, brain development, cognition, male fertility,[5] also there is increasing evidence for the association of OSN in the regulation of atherosclerotic vascular disease.[6,7] Osteocalcin enhance the male fertility; it performs this endocrine function by binding to a G protein-coupled receptor expressed in the Leydig cells of the testes; it regulates the expression of enzymes that are required for testosterone synthesis, promoting germ cell survival. So OSN regulates male fertility through its role in testosterone synthesis.[8] Hormonal regulation of male reproduction involves the release of hypothalamic gonadotrophin-releasing hormone, which in turn stimulates pituitary gonadotrophins that support spermatogenesis, and testicular testosterone production.[9] A gonadotropin-releasing hormone secreted by the hypothalamus elicits the release of gonadotrophins i.e., FSH and luteinizing hormone (LH) from the pituitary gland.[10] FSH binds with receptors in the Sertoli cells and stimulates spermatogenesis, LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis.[11] FSH and LH target the gonads and regulate the secretion of steroid hormones.[12] The failure of the pituitary to secret FSH and LH will result in disruption of testicular function leading to infertility. Abnormal spermatogenesis is often associated with altered serum gonadotropins and testosterone, which is required for successful completion of the spermatogenesis process.[13] PROL hormone secreted from the anterior pituitary hurts male fertility if higher than its physiological level.[14]

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Elevated levels of serum prolactin have a detrimental impact on male reproduction through inhibition of the pulsatile release of gonadotrophins from the anterior pituitary gland, and a direct effect on spermatogenesis.[15]

MATERIALS AND METHODS

The study was performed at the National Center of Teaching laboratories of Medical city institute in Baghdad. The Ethics Committee approved the study of the University of Baghdad, Faculty of Pharmacy (IEC registration no. A2018-10) . Forty two newly diagnosed infertile males age range (24-47) years with a mean age (33 ± 0.893) and thirty-two healthy males as controls age range (25-58) years with a mean age (37.00 ± 1.737) . Serum levels of TEST, LH, FSH, and PROL were performed in addition to serum level of OSN for both patients and controls, FBS also estimated for both groups to exclude the effect of diabetes from the current study. Estimation of serum OSN by Immulite1000 auto-analyzer, TEST, FSH, LH, PRL, and FBS by Immulite2000 auto-analyzer, the company name, and catalog number for each estimated variables are shown in Table 1.

Infertile patients have elevated serum levels for FSH (2.561 ± 0.110), LH (2.188 ± 0.113), PROL (11.80 ± 0.820) and FBS (99.09 ± 0.957) as compared with controls: (1.90 ± 0.115), (1.74 ± 0.210), (7.12 ± 0.287) and (94.59 ± 1.373) respectively. on the other hand, the infertile patients have lowered serum levels for OSN (3.40 ± 0.283) and Testo (228.976 ± 6.216) as compared with controls: (12.70 ± 1.061) and (385.181 ± 13.554) respectively. FSH, LH, and PROL show a significant increase for patient serum samples with P value (0.000*), (0.044*), and (0.000*), respectively. OSN and TEST show a significant decrease for patient serum samples with p value (0.000*) and (0.000*), respectively. FBS is evaluated within the normal levels for both patients and control groups to exclude the effect of diabetes on the current study.

Table 1: Company name and catalog number for the estimated variables

Test name	*Company name*	*Catalog no.*
Osteocalcin	Siemens USA	026L
Testosterone	Siemens USA	L2KTW2
Follicle Stimulating hormone	Siemens USA	L2KFS2
Leutenizing hormone	Siemens USA	348
Prolactin	Siemens USA	344
Blood Sugar	Siemens USA	EA9261
Substrates	Siemens USA	1236

Statistical analysis

An independent t-test was performed to assess significant differences between means. $p < 0.05$ was considered statistically significant. The receiver operation characteristic curve was used to identify the validity of markers as an indicator of infertility in males. The markers were compared according to the area under a curve. An independent t-test was performed to assess significant differences for hormonal evaluation, $p < 0.05$ was considered statistically significant.

RESULTS

The significant differences between means of both infertile patients and controls for hormonal evaluation shown in Table 2.

The receiver operation characteristic curve was used to identify the validity of markers as an indicator of infertility in males. The markers were compared according to the area under the curve, as shown in Figure 1.

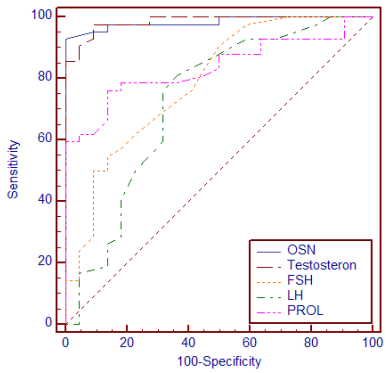

Figure 1: Area under the curve, for serum OSN, TEST, FSH, LH, PROL

Table 2: Hormonal valuation for both infertile patients and controls

Variables	*Group*	*N*	*Mean*	*Std. deviation*	*Std. error*	*p-value*
Age years	Control	32	37.000	8.14745	1.73704	0.02*
	Patients	42	33	5.787	0.893	
OSN (ng/mL)	Control	32	12.7000	4.97766	1.06124	0.000*
	Patients	42	3.407	1.835	0.283	
TEST (ng/dL)	Control	32	385.181	63.574	13.554	0.000*
	Patients	42	228.976	40.284	6.216	
FSH (MIU/mL)	Control	32	1.9000	.54336	.11584	0.000*
	Patients	42	2.561	0.716	0.110	
LH (MIU/mL)	Control	32	1.7409	.98593	.21020	0.044*
	Patients	42	2.188	0.734	0.113	
PROL (ng/mL)	Control	32	7.1273	1.34666	.28711	0.000*
	Patients	42	11.8000	5.317	0.820	
FBS mg/dL	Control	32	94.5909	6.44138	1.37331	0.008*
	Patients	42	99.0952	6.203	0.95716	

** p <0.05 was considered statistically significant.*

Area under the curve, standard error, and 95% confidence intervals for the variable in Table 3.

The sensitivity, specificity, and cut off point of the variables as shown in Table 4.

The pairwise comparison of ROC curves shows the significance level between the estimated variables: Table 5.

Area under the curve reveal that, next to TEST (Figure 2) the main fundamental biomarker for male infertility, OSN (Figure 3) with a high AUC and CL (Table 3), also the high sensitivity and specificity (Table 4) if compare with other variables, so it considered as the best biomarker for detection of male infertility.

DISCUSSION

The skeleton exhibits special role as target tissue for a number of systemic hormones in the human body, in addition to its action as an endocrine tissue targeting a number of extra-skeletal systems. Osteoblasts express OSN, which modulates beta cells of the pancreatic islet, brain, muscle, adipose tissue and testes through G protein-coupled receptor family C group 6member A (GPRC6A) receptors.[16] Osteocalcin functions both locally in bone and as a hormone and this depends on the post-translational mechanism that alters OSN's affinity for the bone matrix and bioavailability.[17]

The role of OSN in the regulation of endocrine effects has been evaluated in a number of recent studies.[18] The detection of OSN as a regulator of testosterone production reflecting its association with male infertility; it plays a role through promoting testosterone biosynthesis, so regulates reproductive action and sperm production in men.[19] Following its binding GPRC6A expressed in Leydig cells,[20] OSN promotes cAMP production that activates the factor cAMP response element-binding (CREB). CREB activates the expression of the genes that are responsible for encoding the essential enzymes that are needed for testosterone biosynthesis, such as StAR, Cyp11a, 3-HSD and Cyp17.[21] In the current study, the infertile patients show a significant decrease of TEST and OSN serum levels when compared with the controls and a significant increase of both FSH, LH, and PROL when compared with the controls. Serum levels of total OSN were positively associated with serum level of testosterone.[22,23] So OSN deficiency could be hypothetically considered a new pathogenic factor responsible for primary testicular failure in men.[16] The significant elevation of FSH and LH blood levels in the presence of low testosterone levels correlate with primary hypogonadism. So the evaluation of TEST, FSH, and LH is useful for the management of male

Table 3: Area under the curve, standard error and 95% confidence intervals for serum OSN, TEST, FSH, LH and PROL

Variable	*AUC*	*SE* [a]	*95% CI* [b]
OSN	0.984	0.0132	0.915 to 1.000
TEST	0.985	0.0107	0.918 to 1.000
FSH	0.788	0.0613	0.668 to 0.880
LH	0.728	0.0730	0.602 to 0.832
PROL	0.836	0.0492	0.722 to 0.917

a. Hanley & McNeil. 1982 *b. Binomial exact*

Table 4: Sensitivity, Specificity, and cut off point of serum OSN, TEST, FSH, LH, and PROL infertile patients:

Variables	*Sensitivity*	*Specificity*	*Cut off point*
OSN	92.9	100.0	≤ 5.9
TEST	97.6	90.9	≤310
FSH	54.8	86.4	> 2.3
LH	81.0	63.6	> 1.5
PROL	76.2	86.4	> 8.6

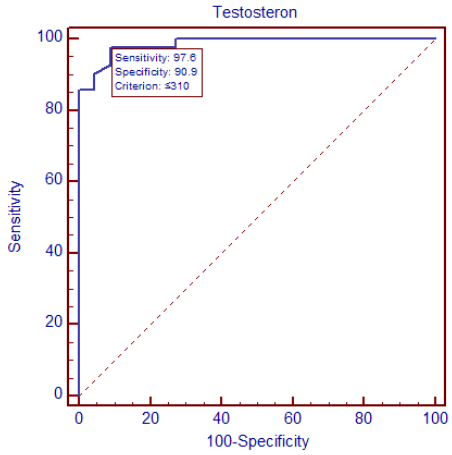

Figure 2: Area under the curve for TEST

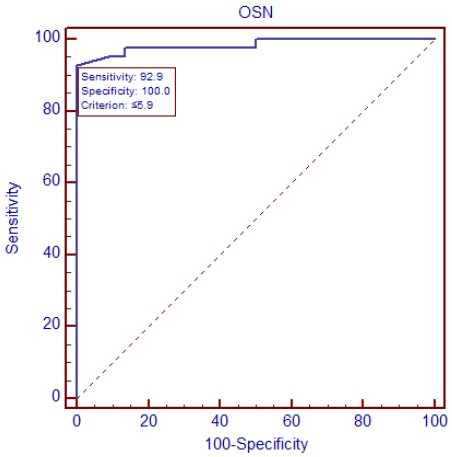

Figure 3: Area under the curve for OSN

Table 5: Pairwise comparison of ROC curves

Comparison between parameters	*p-value*
OSN ~ TEST	0.9237
OSN ~ FSH	0.0015*
OSN ~ LH	0.0005*
OSN ~ PROL	0.0019*
TEST ~ FSH	0.0013*
TEST ~ LH	0.0004*
TEST ~ PROL	0.0028*
FSH ~ LH	0.4875
FSH ~ PROL	0.5126
LH ~ PROL	0.1810

* $p < 0.05$ was considered statistically significant

infertility.[24] Hyperprolactinemia repress testosterone synthesis and male fertility, both directly and indirectly, has a negative effect on sperm production, and its detection and controlling in men seeking fertility is required.[15] In the current study, the estimation of prolactin shows a significant increase for infertile patients if compared with controls, and this reflects the role of it on infertility. Finally: all the estimated hormones, including the skeletal hormone OSN, gonadal hormones FSH and LH, TEST, in addition to prolactin affecting male infertility on different levels. In addition to TEST, the main fundamental biomarker for male infertility is OSN, which has high AUC and CL; also the high sensitivity and specificity if compare with other variables, so it considers as a reliable biomarker for detection of male infertility.

CONCLUSION

The skeleton shows an exceptional role in the human body, as it is a target tissue for many systemic hormones, also it acts as an endocrine tissue targeting a number of extra-skeletal systems. Osteocalcin was positively correlated with serum testosterone, and this correlation ultimately proves the assumption that serum OSN involved in the regulation of sex hormone, in other words, it acts as a regulator of male fertility. Therefore, OSN could serve as a therapeutic for the treatment of disorders related to male reproduction, including male infertility, low sperm counts, and low testosterone levels, and may also serve as a biomarker for evaluation of male infertility.

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